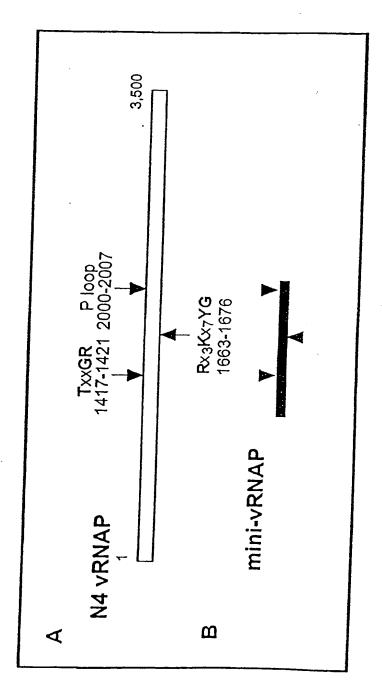
## pest Available Copy

# Bacteriophage N4 vRNAP promoters

G:C C:G X:X' X:X' 3' C:G A A A/T A C C 5

FIG. 2

N4 vRNAP and generation of mini-vRNAP



>

Identification of the minimal active domain of N4 vRNAP

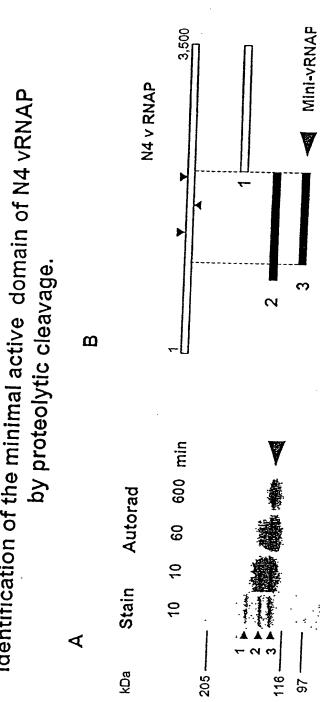
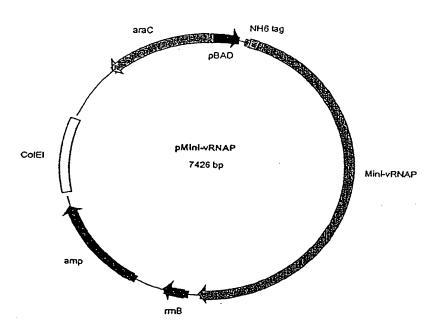


FIG. 4



Plasmid name: pMini-vRNAP
Plasmid slze: 7426 bp
Constructed by: K. M. Kazmierczak
Construction date: 2/2000
Comments: Insert cloned into Invitrogen
pBAD B expression plasmid

FIG. 5

## Purification of cloned vRNAP and mini-vRNAP

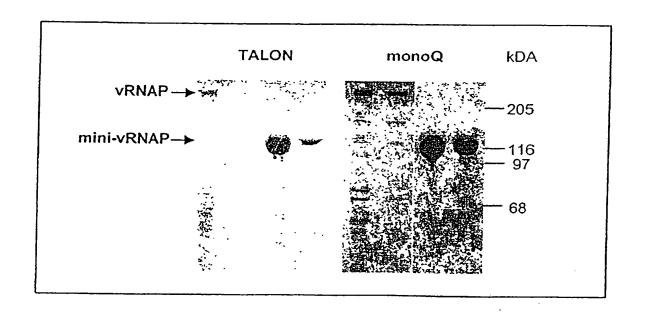
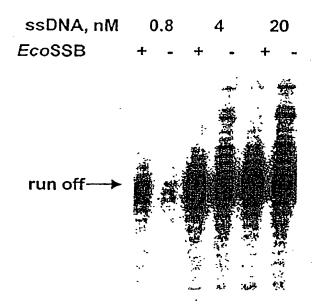


FIG. 6

# Activation of N4 vRNAP transcription by *Eco* SSB at different ssDNA concentrations



4

**FIG. 7** 

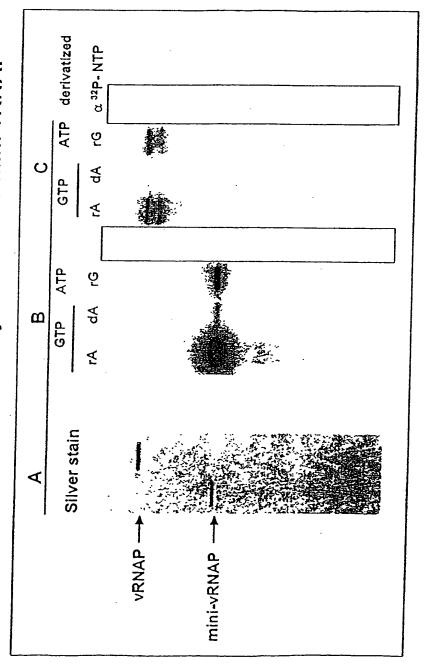
Effect of Eco SSB on ssDNA template recycling

)		,			
•	۵		+	10, 20, 30, 40,	章: : 25:
•	U		+ + +	10, 20, 30, 40,	
	മ	+	; ;	10' 20' 30' 40'	1
	4		; ;	10' 20' 30' 40'	<b>建</b>
		Extra ssDNA	Eco SSB	time at 37 IC	·

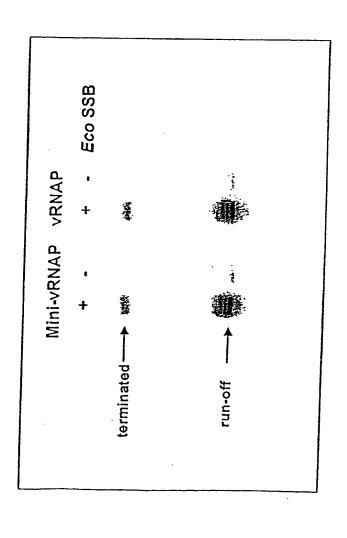
Effect of Eco SSB on the state of template DNA and product RNA in vRNAP transcription

	Eco SSB, 1 µM	H S1 Nuclease	← DNA:SSB ← RNA/DNA:SSB	RNA/DNA
A Z	+	- HS1		
32P DNA	1	- H S1		0
32P RNA	+	- H S1		
32p		- H S1		
	· · · · ·	•	RNA/DNA:SSB — RNA:SSB — 2x(RNA/DNA) —	1x(RNA/DNA)

Transcription initiation by vRNAP and mini-vRNAP



Effect of Eco SSB on transcription of vRNAP and mini-RNAP

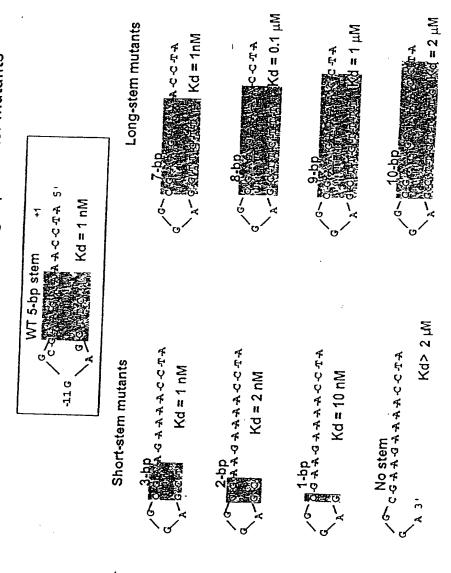


# Determination of mini-vRNAP promoter contacts

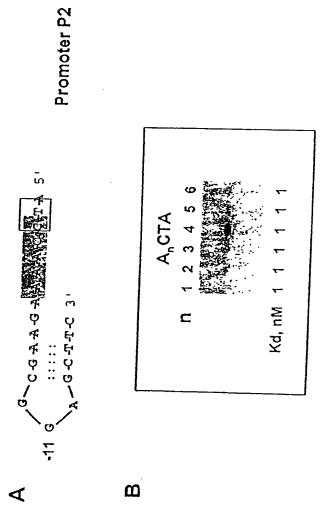
Promoter P2 Kd = 1 nM\s #PP#######PD\B , G C T T C 3' 44 4 Ω

7 Ŧ ņ က္ ņ ထု ~ တု -17 -16 -15 -14 -13 -12 -11 -10 2 150 2 S 9 ~ 312 nm crosslink position Kd, nM

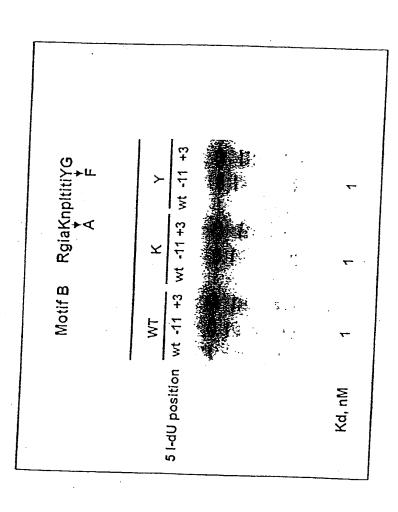
Binding affinities of stem-length promoter mutants



Identification of the transcription start site by catalytic autolabeling



UV crosslinking of mutant mini-vRNAPases to promoter oligonucleotides



Run-off transcription by mutant mini-vRNAPases

		Eco SSB	
	plate ✓+ F	-   +	
	Limiting template WT	+	4
ltitiYG ★	Limiti WT		
RgiaKnpltitiYG ★ A F	)late Y≯F	0	. 8
Motif B	Excess template  WT   K►A   Y►F	0	
Σ	Exce	α 32 P.ATP r d	run-off →

# Mutant mini-vRNAPases transcription initiation

Motif B RgiaKnpltitiYG

★

A

F

K\*A Y\*F

WT K►A Y►F

0.06 0.25 0.06 0.25

FIG. 17

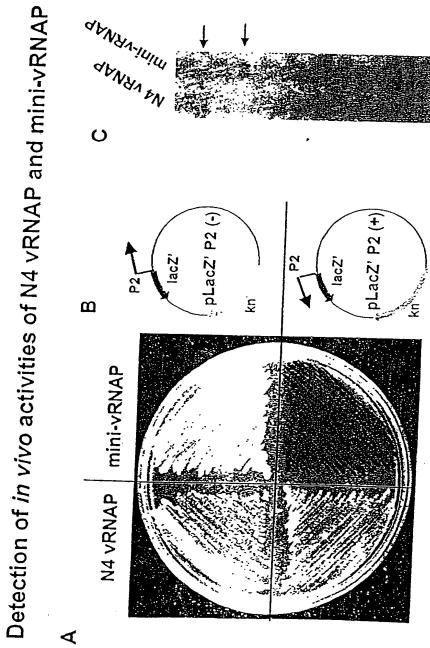


FIG. 18

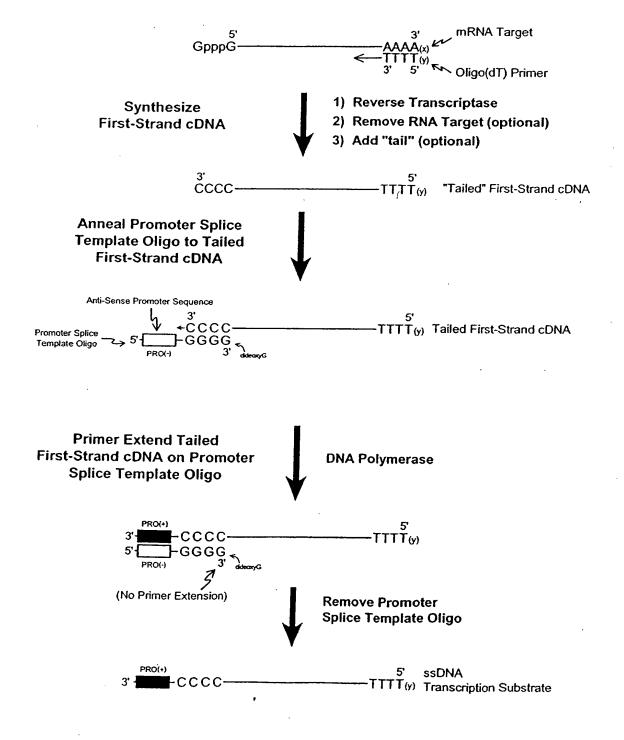


FIG. 19

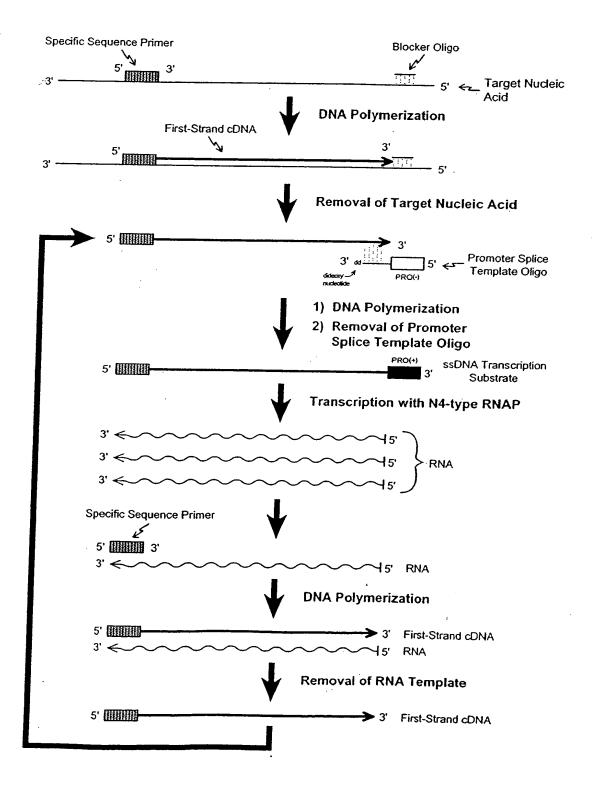


FIG. 20

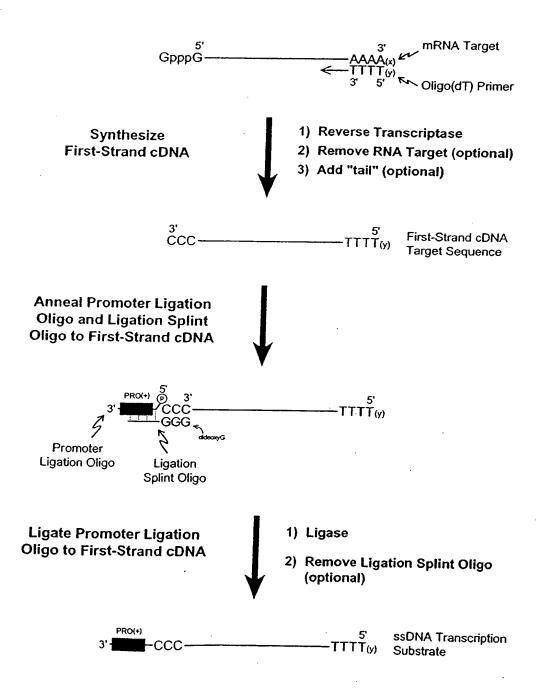


FIG. 21

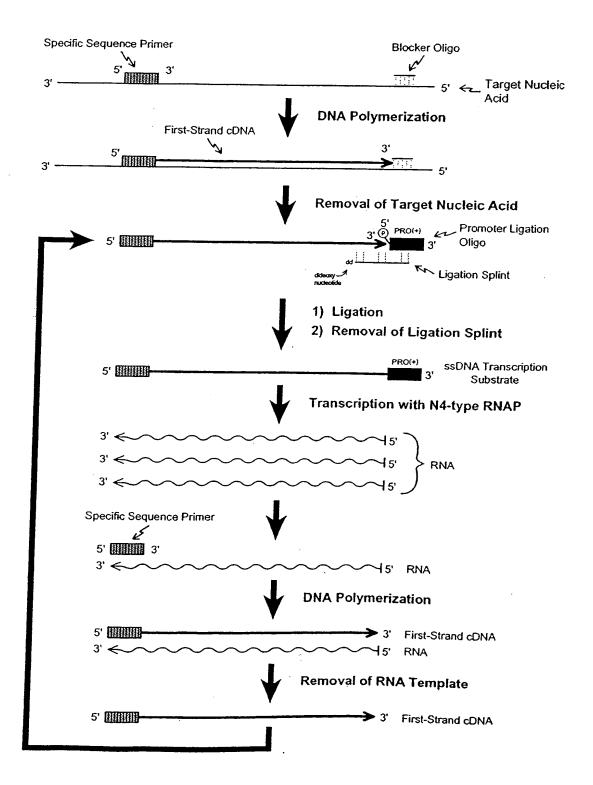


FIG. 22

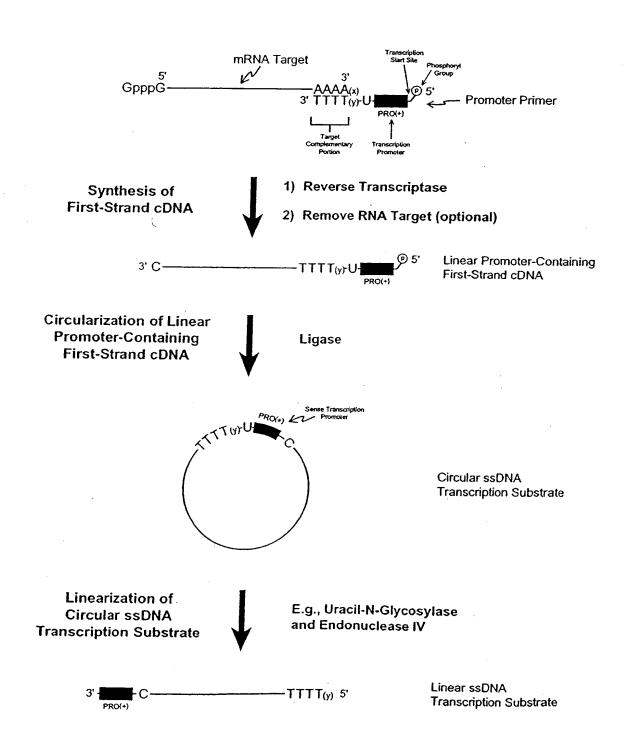
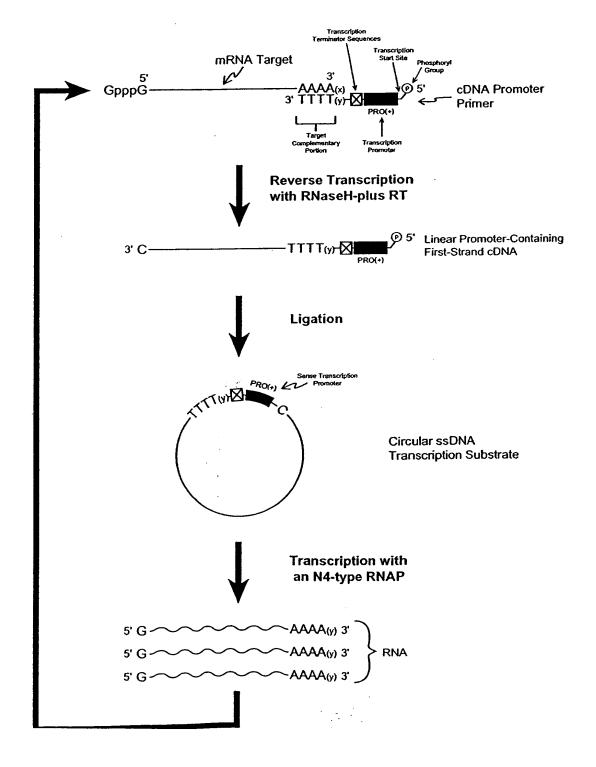
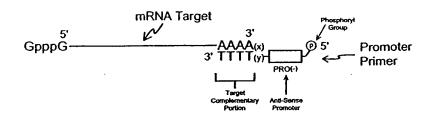


FIG. 23



## FIG. 24 A



Synthesis of First-Strand cDNA



- 1) Reverse Transcriptase
- 2) Remove Target Nucleic Acid

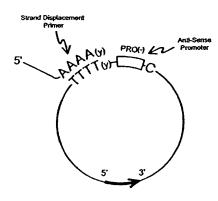


Linear Anti-Sense Promoter-Containing First-Strand cDNA

Circularization of Linear Anti-Sense Promoter-Containing First-Strand cDNA



Ligase



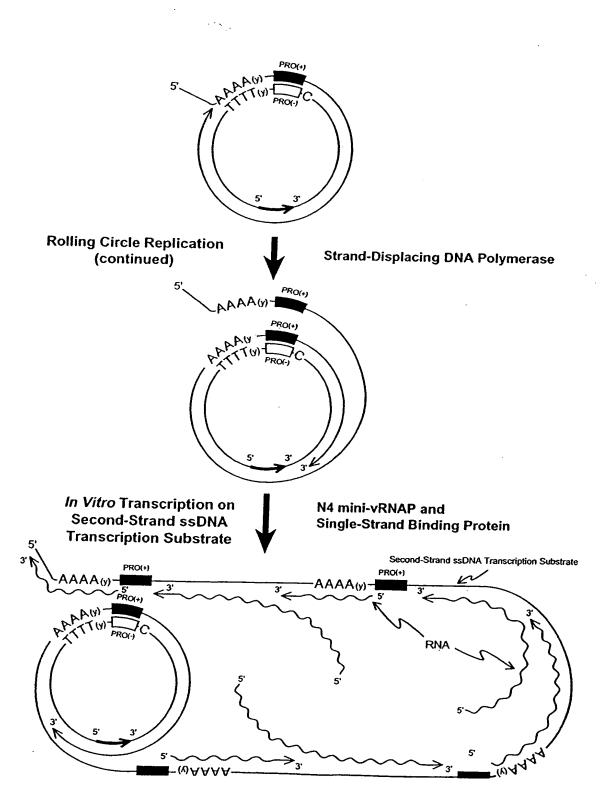
Circular Anti-Sense Promoter-Containing First-Strand cDNA

Rolling Circle Replication



Strand-Displacing DNA Polymerase

FIG. 24 B



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